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GENETICS - On the mechanism of transfer of genetic material in the course of recombination with Escherichia coli K 12. Note of Elie L. Wollman and Francois Jacob, presented by Jacques Trefouel.

In a cross between bacteria Hfr and F-, the transfer of genetic characteristics of the parent Hfr which penetrate the F- bacteria takes place in a determined order. This passage is slow enough so that mechanical treatment applied at various times permits sectioning the chromosomatic segment bearing the characteristics and regulating thus the distribution, among recombinants, of transmitted characteristics.

The high frequency of recombination observed between bacteria Hfr and F- has a bearing only on certain genetic characteristics (1) which are, in order: $TLAzT_1Lac_1Gal_1ly^S$ (2) (synthesis of threonine T, of leucine L, sensibility to nitride of sodium Az, to phage T_1, utilization of lactose Lac of galactose Gal, lysogenic λ ly λ). Everything occurs as if these characters were situated on a segment limited by a point of preferential rupture R, the characteristics situated beyond R, such as S (streptomycin) being transmitted at lower frequency (10^{-5} to 10^{-6}). In a cross using some non-lysogenic bacteria $HfrT^+L^+Az^ST_1^SLac^+Gal_1^+S^S$ and $F^-T^-L^-Az^rT_1^rLac^-Gal_1^-S^r$ one can follow, as a function of the time of contact between Hfr and F-, the evolution of a number of recombinants receiving from Hfr characteristics relatively rare such as T L and Gal .

This number grows linearly as a function of time (fig. A) to attain, toward the 80th minute, a plateau which, for the selection T L S (curve 1), represents about 10% of the initial number of Hfr and only 2,5% for the selection Gal S (curve 3). This difference and that which reveals the genetic analysis of recombinants (25% of T L S are Gal since 80% of the Gal S are T L) indicate an asymmetry of the recombination of segment TL-Gal.

In order to define the kinetics of the recombination, bacteria in process of conjugation have been, at different times, submitted to forces of friction in a high speed homogenizor, treatment which does not affect the viability of the bacteria (3). After mechanical treatment, the appearance of recombinants is retarded (fig. A). Recombinants $T^+L^+S^-$ (curve 2) begin to appear only in samples treated for 10 mn., recombinants Gal^-S^- after about 25 mn. (curve 5). The number of recombinants increases rapidly to attain in about 50 mn. the same level as the controls. Transfer of genetic characteristics from parent Hfr to F- is then distributed within the time, the passage of T^+L^+ being earlier than that of Gal^-.

This progressive transfer appears more clearly still if one compares the genetic constitution of recombinants $T^+L^+S^-$ according to whether they come from samples taken at variable times and whether submitted or not to mechanical treatment. In the absence of treatment, this genetic constitution remains constant, whatever the time of sampling. For 100 recombinants $T^+L^+S^-$ one always finds characteristics issued from parent Hfr in similar proportions: As 90%, T 75%, Lac^- 40%, Gal^+ 25%. After mechanical treatment these proportions

vary as a function of the time of application of treatment, the characteristics of parent Hfr appearing among the recombinants T L S in the order of their liaison to TL (fig. B). After 50 mn. the recombinants arising from treated samples have the same genetic constitution as recombinants of control samples. One can, therefore, in some fashion, set up a genetic chart, in units of time.

From those experiments the following conclusions can be drawn:

1. The segment of chromosome of bacteria Hfr on which bears the high frequency of recombination is a segment oriented O---R, the order of transmission of characteristics being a function of their distance from the unknown origin O. The probability, for a given characteristic, of appearing among the recombinants is much weaker, the farther removed it is from origin O.

2. There can be genetic recombination when only a small fragment of chromosome of parent Hfr is transmitted to an F- bacteria, which corresponds to the conception of Hayes (1). As to the mechanism of integration of genetic material transmitted, recombination with E. coli K 12 seems therefore that it could be aligned with transduction. ( )

References

(1) W. Hayes, Cold Spring Harb. Symp., 18, 1953, p. 75.

(2) E.L. Wollman and F. Jacob, Comptes rendus, 239, 1954, p. 455.

(3) T.F. Anderson, Bot. Rev., 15, 1949, p. 477.

(4) N.D. Zinder and J. Lederberg, J. Bact. 64, 1952, p. 679.

FIGURES

A mixture in bouillon of bacteria Hfr (10^7/ml) and F- (5.10^8/ml) in process of exponential growth is prepared at time 0 and agitated at 37°. Samples are taken at various times, diluted, a portion being submitted to mechanical treatment, the other saved as control. Upon sampling each portion inoculations are made on selective media.

Fig. A - Frequency, as a function of time, of recombinants T L S (before treatment, curve 1); after treatment, curve 2) and Gal S (before treatment, curve 3; after treatment, curve 4).

Fig. B - Genetic analysis of recombinants T L S obtained at the beginning of sampling submitted to mechanical treatment. At each of the times indicated, 120 recombinants have been examined. Distribution of the characteristics issued from parent Hfr is expressed as a function of time at which the samples have been taken off.